Attorney Docket No: 2006636-0064

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellants:	Sontheimer *et al.*	Examiner:	Chen, Shin Lin
Serial No.:	10/686,782	Art Unit:	1632
Filing Date:	October 17, 2003	Confirmation No.:	7705
Title:	Diagnosis and Treatment of Neuroectodermal Tumors		

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Madam:

APPEAL BRIEF UNDER 37 C.F.R. § 41.37

Appellants appeal to the Board of Patent Appeals and Interferences (the "Board") from the Examiner's rejection of claims 1, 15-20, and 22-24. A Notice to this effect was filed pursuant to 37 C.F.R. § 41.31 on January 23, 2009. The Notice was filed electronically at www.uspto.gov and Appellants received an Electronic Acknowledgement Receipt indicating that the Notice was received by the Patent and Trademark Office on January 23, 2009.

A credit card payment of **$270.00** for the fee for a small entity under 37 C.F.R. § 41.20(b)(2) for the Appeal Brief is being filed herewith using the USPTO's Electronic Filing System. Also filed herewith is a Petition under 37 C.F.R. § 1.136 for a one (1) month extension of time, from March 23, 2009, up to and including April 23, 2009, to file this Appeal Brief. A credit card payment of **$65.00** for the fee for a small entity under 37 C.F.R. § 1.17(a)(1) for the Petition is being filed herewith using the USPTO's Electronic Filing System. Therefore, this Appeal Brief is timely filed on March 31, 2009.

A Table of Contents is provided for the convenience of the Board on page **2**.

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Real parties in Interest

As a result of assignments by the inventors in the parent application U.S. Serial No. 09/296,031 (filed April 21, 1999), the real party in interest in this application is UAB Research Foundation. ("UAB"). The assignments to UAB were recorded in the Patent and Trademark Office at Reel 011412, Frame 0154. UAB has licensed the subject matter of this application to TransMolecular, Inc. and to the National Institutes of Health (NIH), U.S. Department of Health and Human Services (DSHS), U.S. Government. The confirmatory license to the National Institutes of Health was recorded in the Patent and Trademark Office at Reel 020929, Frame 0498.

Related Appeals and Interferences

No other appeals or interferences are known to Appellants, Appellants' legal representative, or Appellants' assignee that will directly affect or be directly affected by the Board's decision in this appeal. Similarly, no such appeals or interferences are known that may have a bearing on the Board's decision in this appeal.

Status of Claims

Twenty-eight (28) claims have been filed in this case. Claims 2-14, 21 and 25-28 been canceled.

Claims 1, 15-20, and 22-24 were rejected in Office Actions mailed March 28, 2006, November 21, 2006 (final rejection), December 31, 2007, April 21, 2008 (final rejection), and October 29, 2008.

As of the Office Action issued October 29, 2008, claims 1, 15-20, and 22-24 stand rejected. As addressed below, Applicants filed a Supplemental Response amending claims 17 and 24 on March 30, 2009. Assuming entrance of the Amendment, the status of claims 17 and 24 is not expected to change because the Amendment addresses only typographical errors.

The rejection of claims 1, 15-20, and 22-24 is hereby appealed. A listing of pending claims 1, 15-20, and 22-24 is provided in Claims appendix A (as submitted in the Supplemental Response) beginning on page 24 and Claims appendix B (assuming entrance of the Amendment) beginning on page 26.

Status of Amendments

A response to the non-final Office Action mailed October 29, 2008 was filed on January 23, 2008; no amendments were contained in the response. Appellants filed a Supplemental Response containing amendments to claims 17 and 24 on March 30, 2009. Appellants submit that the amendments in the Supplemental Response are limited to correction of typographical errors of claim dependencies and therefore are in compliance with 37 C.F.R. § 1.111(a)(2).

These amendments have not yet been entered by the Examiner. Claims appendix A presents the claims as they were submitted in the Supplemental Response filed on March 30, 2009. Claims appendix B presents the claims as they would be assuming entrance of the Amendments filed on March 30, 2009.

Summary of Claimed Subject Matter

Chlorotoxin is a 36 amino acid peptide originally isolated from *Leiurus quinquestriatus* scorpion venom. Chlorotoxin has been demonstrated to bind specifically to glial-derived or meningioma-derived tumor cells. The present invention encompasses the discovery that chlorotoxin also recognizes other neuroectodermal tumors and thus can be used to target the whole class of neuroectodermal tumors.

Independent claim 1 and dependent claims 15-20 and 22-24 relate to methods of delivering a cytotoxic moiety to a neuroectodermal tumor comprising: administering a composition comprising an agent consisting of chlorotoxin fused to a cytotoxic moiety to an individual having a neuroectodermal tumor, such that the agent binds specifically to the tumor. Claim 15 is drawn to embodiments in which the chlorotoxin is fused to a particular cytotoxic moiety. Claim 16 is drawn to embodiments in which the neuroectodermal tumor is one of particular types of neuroectodermal tumors. Claim 17 is drawn to embodiments wherein the chlorotoxin is native chlorotoxin, synthetic chlorotoxin, or recombinant chlorotoxin. Claims 18-19 depend from claim 17 and are drawn to embodiments in which the neuroectodermal tumor is a glioma. Claim 20 depends from claim 17 and is drawn to embodiments in which the neuroectodermal tumor is one of particular types of neuroectodermal tumors. Claim 22 is drawn to embodiments in which the composition further comprises a pharmaceutically acceptable carrier. Claims 23-24 are drawn to embodiments in which the composition is suitable for parenteral administration.

Ground of Rejection to be Reviewed on Appeal

The ground of rejection to be reviewed on appeal is (referring to §§ **2-3** of the Office Action mailed October 28, 2008):

(1) whether claims 1, 15-20, and 22-24 are enabled under 35 U.S.C. § 112, first paragraph.

Grouping of Claims

For the reasons discussed below in the Argument section, the claims stand or fall together for purposes of ground of rejection numbered (1) above, as indicated below:

(1) Claims 1, 15-17, 20, and 22-24 stand or fall together.

(2) Claims 18 and 19 stand or fall together.

Argument

Introduction

Appellants must admit some frustration with the prosecution of this case. There have only ever been two rejections (and one provisional rejection) levied during the entire prosecution, one of which was addressed trivially in the first Office Action. The second rejection, for lack of enablement, has been maintained through *five* Office Actions, but for ever-changing reasons. Appellants have endeavored to work with the Examiner to address his concerns, including having both in-person and telephonic interviews during which the Examiner indicated that proposed amendments or evidence would indeed be helpful, and also providing extensive declaratory evidence. Unfortunately, it seems impossible for Appellants to succeed in overcoming the rejection because, each time he levies it, the Examiner gives different reasons for his concerns. Each time, Appellants address the articulated concern but Examiner maintains the rejection, now for a new reason. Moreover, the most recent Office Action appears to revert to an earlier articulation of the Examiner's concern, which Appellants understood they had previously addressed and resolved.

Appellants below summarize the entire prosecution history of this case, in order to illustrate the evolving nature of the maintained rejection, as well as the thoroughness of Appellants' responses to each articulation of the rejection. Later, in a final effort to advance prosecution of this case, Appellants present a complete response to *every* version of the lack of enablement rejection levied by the Examiner. As will be clear, Appellants have more than satisfied the legal requirements for enablement, the present claims are patentable to Appellants, and the rejection should be reversed.

Claims 1, 15-17, 20 and 22-24 satisfy the enablement requirement under 35 U.S.C. § 112, first paragraph

The presently pending claims relate to a method of delivering a cytotoxic moiety to a neuroectodermal tumor, comprising: administering a composition comprising an agent consisting

of chlorotoxin fused to a cytotoxic moiety to an individual having a neuroectodermal tumor, such that the agent binds specifically to the tumor.

The specification includes data from experiments showing the specific binding of synthetic chlorotoxin (TM-601) to tissues from 18 different neuroectodermally derived tumors. Appellants have also provided declaratory evidence demonstrating clinical uptake of chlorotoxin delivered either intracranially or intravenously to subjects suffering from neuroectodermally derived tumors (including gliomas, melanomas, *etc*). The present Appellants discovered, among other things, that chlorotoxin-derived molecules can be used to specifically target neuroectodermal tumors for therapeutic and/or diagnostic purposes, and that conjugates of chlorotoxin linked to cytotoxic moieties can be employed for such purposes. The specification enables these findings, as recited in the present claims.

The initially examined claims referred to a method of treating an individual having a neuroectodermal tumor, comprising administering a pharmaceutical composition comprising an effective dose of chlorotoxin fused to a cytotoxic moiety.

In an Office Action mailed on March 28, 2006 ("the first Office Action") the Examiner issued a rejection under 35 U.S.C. § 112, second paragraph for supposed incompleteness and a rejection under 35 U.S.C. § 112, first paragraph for supposed lack of enablement. The incompleteness rejection was addressed easily and will not be discussed further here.

As regards the enablement rejection, the Examiner alleged that "the specification fails to provide adequate guidance and evidence for how to treat a neuroectodermal tumor. . . by using a pharmaceutical composition comprising a chlorotoxin fused to a cytotoxic moiety. . . via various administration routes in vivo" because the claims read on protein therapy *in vivo* and the Examiner argued that protein therapy was supposedly unpredictable at the time of the invention. We note, however, that, in laying out this argument, the Examiner cited articles discussing *gene* therapy.

The Examiner's specific challenge to the claims, as set forth in the first Office Action, was that several neuroectodermal tumors are located in the brain and, according to the Examiner, the blood-brain barrier would present a challenge for gene delivery and protein delivery to such

tumors inside the brain. Additional comments by the Examiner in the first Office Action related to difficulties in predicting function from a protein's structure.

Appellants therefore understood that the claims were rejected for lack of enablement because, according to the Examiner, (1) protein therapy in general is unpredictable; and (2) whether chlorotoxin fusions would cross the blood-brain barrier was unpredictable. Appellants filed a response addressing these points on September 14, 2006.

To address the Examiner's comments regarding the alleged unpredictability of protein therapies, Appellants cited several examples of protein therapeutics that have been approved for use for many years well before the filing date of the present application. Appellants further pointed out that problems with gene therapy are not directly translatable to problems with protein therapy.

To address the Examiner's comments regarding the blood-brain barrier, Appellants submitted evidence (including a scientific article) that chlorotoxin fusions can be effectively and specifically delivered across the blood-brain barrier to brain tumor sites. Appellants respectfully pointed out that the Examiner's comments with respect to prediction of protein function from protein structure were misplaced.

In a Final Office Action mailed November 21, 2006 ("the second Office Action"), the Examiner maintained the enablement rejection, but did not explain why Appellants' arguments had not resolved his concern. The Examiner simply stated that Appellants' arguments were "not found persuasive because of the reasons set forth in the preceding Office Action mailed 3-28-06."

The Examiner dismissed the proffered evidence of chlorotoxin fusions crossing the blood-brain barrier by merely stating "it is still unclear whether chlorotoxin-cytotoxic moiety complex can pass through blood brain barrier in a subject", without explaining why it was "still unclear".

The Examiner also repeated his assertion that "the art or [sic] protein therapy was unpredictable at the time of the invention", without commenting on the many approved

therapies, and repeated verbatim his comments regarding protein therapy from the first Office Action.

Thus, although Appellants had addressed the concerns stated in the first Office Action, the Examiner was not satisfied. The Examiner offered an apparently new basis for the enablement rejection by asserting that "treatment of different neuroectodermal tumors with different cytotoxic moieties has to be considered individually" and alleged that "one skilled in the art at the time of the invention would require undue experimentation to practice over the full scope of the invention claimed."

Appellants now understood that the basis for the lack of enablement rejection levied by the Examiner was that the Examiner required evidence of successful treatment of *each* different kind of neuroectodermal tumor encompassed by the claims, and *each* different kind of cytotoxic moiety. Appellants therefore filed a response along with a Notice of Appeal on May 21, 2007 explaining that the current legal standards for enablement for method of treatment claims, as articulated by *In re Brana*, do not require such evidence. Appellants pointed out and summarized the data provided by the present application, which, as explained below, includes human clinical data and supports methods of treatment of a variety of neuroectodermal tumors using a variety of cytotoxic moieties via a variety of routes of administration. Appellants further provided a Declaration confirming that chlorotoxin fusions do in fact bind specifically to a variety of tumors (including gliomas and melanomas) when delivered by intracavitary or intravenous administration to humans. Appellants explained that the Specification, as confirmed by the Declaration, fully satisfied the proper legal requirements for enablement.

The Examiner mailed an Advisory Action on June 27, 2007 claiming that the present case was not analogous to *In re Brana* (on the ground that *Brana* related to use of a cytotoxic *agent* whereas the present case related to use of a fusion of a cytotoxic agent with a delivery moiety – respectfully, a distinction without a difference!) and again asserting a lack of enablement.

Appellants endeavored to better understand the Examiner's position by requesting an in-person interview. In that interview, on August 27, 2007, the Examiner indicated that the

enablement rejection related to his concern for a supposed lack of sufficient evidence of successful *treatment*, but that he did not question the evidence that chlorotoxin fusions could be specifically *delivered* to tumors. The Examiner offered that an amendment reciting "delivery" rather than "treatment" would be helpful.

Appellants therefore filed a response on October 31, 2007. Appellants maintained that the specification is fully enabling for methods of treatment, but amended the claims to methods of delivery in order to advance prosecution of this case.

In an Office Action mailed December 31, 2007 (the third Office Action), the Examiner again maintained the enablement rejection, repeating verbatim the language from the Office Action mailed November 21, 2006 (the second Office Action). In a telephone interview with Appellants' representative on January 16, 2008, the Examiner clarified that he still had concerns that the claims read on methods of treatment rather than on methods of delivery because of the terms "pharmaceutical composition, "effective dose," and "treated." Appellants filed a response on January 24, 2008 amending the claims to remove such terms, in order to advance prosecution of the case.

In an Office Action mailed April 21, 2008 (the fourth Office Action), the Examiner maintained the enablement rejection. The Examiner even made reference to a "pharmaceutical composition" even though Appellants had amended the claims to remove the term. The Examiner acknowledged, however, that the specification is "enabling for delivering chlorotoxin fused to a cytotoxic moiety to neuroectodermal tumors *in vitro* or via intravenous administration or intracavitary injection in the brain *in vivo*..."

Given the confusing reference in the rejection to language no longer present in the claims, and the fact that the Examiner acknowledged enablement with respect to at least two totally different (one local, one systemic) routes of administration, Appellants requested another telephone interview in order to better understand what more could possibly be required in order to overcome the rejection. In a phone interview with Appellants' representative on July 21, 2008, the Examiner then indicated that he had returned to his concern that the claims encompassed different types of tumors. This time, however, the Examiner specifically

mentioned a concern that the claims encompassed delivery to tumors that are *not* located in the brain and that there was a supposed lack of evidence that chlorotoxin can be delivered to different tumors and sites with different administrative routes.

Thus, Appellants had apparently overcome the Examiner's previously-stated concern that chlorotoxin fusions might not cross the blood-brain barrier, but that concern had been replaced with a new concern that chlorotoxin fusions might not bind to tumors that *did not* require crossing the blood brain barrier! Of course the Examiner did not address the previously-provided evidence that intravenously administered chlorotoxin fusions do in fact bind to neuroectodermally derived tumors outside of the brain (*e.g.*, melanomas, as already discussed). Moreover, the Examiner provided no *reason* to justify his stated concern that, in light of the provided evidence that chlorotoxin fusions *do* bind specifically to *various* tumors when administered by *either* local or systemic routes, there might be some reason to doubt that the same would not be achieved with respect to particular other tumors or routes. The Examiner also mentioned a new concern that some cytotoxic moieties might accumulate in the liver.

Appellants filed a response on August 7, 2008 reminding the Examiner, among other things, that (1) the present specification provides evidence of chlorotoxin binding to tumors outside of the brain; (2) Appellants had previously provided evidence that chlorotoxin does not accumulate in the liver; and (3) Appellants had provided evidence that completely different routes of administration are in fact effective, such that there is no reason to doubt the effectiveness of other routes.

In the most recent Office Action mailed October 28, 2008 (the fifth Office Action), the Examiner again maintained the rejection of claims 1, 15-20 and 22-24 under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement. The language used in this Office Action again acknowledges that certain aspects of the claims are enabled, but provides a *more narrow* description of such scope than was presented in the fourth Office Action. Specifically, the fifth Office Action states "the specification, while being enabling for delivering chlorotoxin fused to a radioisotope to neuroectodermal tumors *in vitro* or via intravenous administration or intracavity [sic] injection of brain *in vivo*, does not reasonably provide enablement for delivering various cytotoxic moieties, including protein or nucleic acid,

to a neuroectodermal tumor *in vivo* by administering a composition comprising a chlorotoxin fused to cytotoxic moiety, including, proteins, to an individual via various administration routes…"

Given the repeated morphing of the reasons cited by the Examiner for the enablement rejection, Appellants hereby address all of the arguments made by the Examiner in the current Office Action or in others. As explained below, Appellants have demonstrated that the present application enables delivery of (1) a range of cytotoxic moieties to a (2) range of tumor types (3) via a variety of routes of administration; that furthermore the chlorotoxin-cytotoxic moiety conjugates have a therapeutic effect *in vivo*; and that the data provided in the specification and in the Declaration by Dr. Alison O'Neill under 37 C.F.R § 1.132 filed May 21, 2007 are sufficiently predictive.

Appellants respectfully submit that, as previously acknowledged by the Examiner, the specification is not required under the law to provide enablement for every species encompassed by the claims. Nonetheless, the Examiner is essentially requiring demonstration of every possible combination of cytotoxic moieties, tumor type, and route of administration encompassed by the claims. Appellants submit that this demand is neither reasonable nor consonant with the law. Furthermore, in laying out grounds for the enablement rejection, the Examiner continues to ignore evidence presented by Appellants refuting the Examiner's allegations of lack of enablement.

Cytotoxic moieties

The presently pending claims recite the use of a conjugate consisting of a chlorotoxin agent fused to a cytotoxic moiety (hereafter "chlorotoxin conjugate"). The Examiner has raised issues as to the range of cytotoxic moieties whose delivery via the claimed method is enabled by the present specification. Stating that the claims read on protein therapy *in vivo*, the Examiner questioned the nature of protein therapeutics.

For example, in the first Office Action, the Examiner alleged that "the specification only discloses the detection of glioblastoma, neuroblastoma, medulloblastoma, pheochromocytoma,

and metastatic melanoma *etc.* in a tissue sample by using chlorotoxin." In the fifth Office Action, the Examiner acknowledged that the specification is enabling for a method of delivering chlorotoxin fused to a radioisotope to neuroectodermal tumors. Nevertheless, the Examiner continued to repeat his allegation that the specification is not enabling for methods in which the cytotoxic moiety is a protein.

Contrary to the Examiner's remarks, Appellants have demonstrated successful delivery of a variety of moieties *including proteins*, to neuroectodermal tumors using chlorotoxin. As mentioned in previously filed response, Appellants have demonstrated that the cytotoxic moieties that can be used in accordance with the invention are not limited to radiolabels such as ^{131}I. At least five chlorotoxin complexes (TM-602, *i.e.*, chlorotoxin covalently linked to biotin; ^{131}I-TM-601, *i.e.*, chlorotoxin radiolabeled with ^{131}I; chlorotoxin labeled with ^{125}I; chlorotoxin-GST fusion protein; and chlorotoxin-GST fusion protein attached to saporin) have been tested and have showed neuroectodermal tumor-selective binding and uptake in at least one *in vitro* or *in vivo* system. With regard to delivery of proteins, Appellants have demonstrated that chlorotoxin (which is itself a peptide) when fused to glutathione-S-transferase (GST, a protein) and attached to saporin (another protein) via antibodies (also proteins) is specifically bound and taken up by cancer cells, and effects selective killing of glioma cells. (See, *e.g.*, Example 23 of U.S. Pat. No. 5,905,027, the contents of which the present application incorporates by reference in their entirety.)

Therefore, in contrast to the Examiner's remarks, the specification is enabling for a range of cytotoxic moieties *including proteins.*

Despite Appellants' evidence to the contrary, the Examiner continues to raise issues with protein therapy. Appellants submit that the specification has fully satisfied the enablement requirement as regards to the range of cytotoxic moieties encompassed by the claims.

Tumor types

The Examiner has remarked upon the range of tumor types encompassed by the claims and has alleged that the specification provides enablement for "only" a certain subset of tumor types.

As explained previously, Appellants have presented evidence in the present application that chlorotoxin binds to a variety of tumors, including tumors located both inside and outside the brain. Appellants provided data from experiments on more than 250 frozen or paraffin sections showing the specific binding of synthetic chlorotoxin (TM-601) to tissues from 18 different neuroectodermally derived tumors (*i.e.*, from WHO grade IV: glioblastoma multiformes, WHO grade III: anaplastic astrocytoma, WHO grade II: low grade, WHO grade I: pliocytic astrocytoma, oligodendriomas, other gliomas, gangliomas, meningiomas, ependymomas, metastatic tumors in the brain, medulloblastomas, neuroblastomas, ganglioneuromas, pheochromocytomas, peripheral primitive neuroectodermal tumors, small cell carcinoma of the lung, Ewing's sarcoma, and melanomas (see Examples 8-17 of the application as filed). The afore-mentioned list presents the entire list of tumors recited in claims 19-20. Tumors among the afore-mentioned list that reside outside the brain include pheochromocytomas (located in the adrenal glands near the kidney; see Figure 4 of the specification as originally filed), small cell lung carcinomas (see Figure 9), and Ewing's sarcoma (a bone cancer; see Figure 12).

Appellants have furthermore demonstrated successful *in vivo* targeting to a variety of tumors, including tumors inside the brain. Intracavitary administration of a chlorotoxin conjugate resulted in specific binding and uptake in high grade gliomas. Intravenous administration of a chlorotoxin conjugate resulted in selective uptake in glioma and metastatic melanoma, two of the claimed neuroectodermally-derived tumors. As elaborated below, such targeting was successful even when the chlorotoxin-cytotoxic moiety conjugate was delivered in such a way that required the conjugate to cross the blood-brain barrier.

Routes of administration

The Examiner has taken issue with the range of routes of administration that are encompassed by the claims, and had mentioned the blood-brain barrier as a deterrent for delivery of chlorotoxin conjugates to brain tumors. The Examiner has also argued, essentially, that protein and peptide therapeutics *may* be eliminated from the body before they may confer a therapeutic effect. In the first and subsequent Office Actions, the Examiner asserted that "It is unclear how the chlorotoxin complex would reach the targeted tumor in the brain via oral administration, intravenous administration, intramuscular administration, subcutaneous administration, or intrathecal administration *etc.*, to a subject."

Appellants have demonstrated in *human clinical trials* that chlorotoxin (a peptide) fused to a cytotoxic moiety (in this case, chlorotoxin labeled with ^{131}I) (1) selectively reaches its target tumor site when administered to a patient either through intracranial or intravenous administration; (2) *passes through the blood-brain barrier* to reach a tumor located in the brain; and (3) has a therapeutic effect *in vivo*. (See Declaration by Dr. Alison O'Neill, submitted May 21, 2007 and attached herewith in the Evidence appendix.) That the chlorotoxin-cytotoxic moiety conjugate (which necessarily comprises a peptide, because chlorotoxin is a peptide) has been demonstrated to have a therapeutic effect *in vivo* refutes the Examiner's conjecture that a chlorotoxin conjugate would be eliminated from the body before it elicits a therapeutic effect.

Furthermore, intravenous administration of the chlorotoxin complex was found to result in selective uptake in glioma and metastatic melanoma, two of the claimed neuroectodermally-derived tumors.

The Examiner has continued to argue against enablement for a range of routes of administration by focusing on potential problems with delivery of proteins. Again, the Examiner appears to demand, essentially, that every possible combination of route of administration, cytotoxic moiety, and tumor type encompassed by the claims be explicitly demonstrated in the specification. Appellants reiterate that the specification need not be enabling for every species. Nonetheless, Appellants have demonstrated successful delivery of a chlorotoxin-cytotoxic moiety by two very different routes of administration, including one that

involved successful penetration of the blood-brain barrier. Appellants submit that the positive results with very different routes of administration are more than sufficiently predictive to enable the range of routes of administration encompassed by the claims.

Legal standard for enablement

Appellants have not only met, but also far exceeded, the legal standard for enablement for methods of treatment, let alone for methods of delivering a cytotoxic moiety to a neuroectodermal tumor .[1] As solidified by *In re Brana* (51 F.3d 1560 USPQ2d 1436 (Fed. Cir. 1995), the legal standard for enablement does not require *in vivo* evidence in humans. Accordingly, it is an unusual patent application that is supported by clinical evidence of even one claimed species. The Examiner's demands are excessive and inconsistent with the law.

As Appellants discussed in a response filed May 21, 2007, the Brana court held that

> "proof of an alleged pharmaceutical property for a compound by statistically significant tests with standard experimental animals is sufficient to establish utility." (51 F.3d 1567)

The court explained its holding by stating:

> "We hold as we do because it is our firm conviction that one who has taught the public that a compound exhibits some desirable pharmaceutical property in a standard animal has made a significant and useful contribution to the art, even though it may eventually appear that the compound is without value in the treatment of humans." (51 F.3d 1567)

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[1] To obviate the Examiner's objections to terminology referring to treatment, Appellants had amended the claims to refer to a method of delivery and had removed phrases such as "pharmaceutical composition" that the Examiner deemed to be related to methods of treatment. Nevertheless, Appellants maintain that claims to methods of treatment are fully enabled by the specification.

Even a cursory search on the USPTO's patent full-text and image database for patents related to peptide and/or protein therapies reveals many issued patents whose claims to methods of treatment are not supported by *in vivo* data in humans. Specifications of many patents do not even include any *in vivo* data whatsoever. To give but a few examples, U.S. Pat. No. 6, 171,818 (the '818' patent) was issued on January 9, 2001 and claims a method for treating cancer comprising administering a protein present in sea snails or hares. Exemplification in the '818 patent was limited to isolation of the protein and *in vitro* experiments involving incubating the protein with cell lines. As another example, U.S. Pat. No. 7,112,329 (the '329 patent) was issued on September 26, 2006 and claims a method for treating pollinosis comprising administering a peptide derived from a Japanese pollen allergen molecule. The specification of the '329 patent describes only *in vitro* experiments, for example, synthesis of peptides, expression of recombinant proteins in bacteria, establishment of T-cell lines and antigen-presenting cell lines for use in experiments, and identification of a peptide epitope involved in pollinosis. For both the '818 and '329 patents, no *in vivo* data was presented, let alone data in humans demonstrating any effect whatsoever. Nevertheless, method of treatment claims encompassing treatment of humans were deemed supported by the specification.

Further examples include patents that do provide *in vivo* data, but do not provide any data in humans. For example, U.S. Pat. No. 7,442,681 (the '681 patent) was issued October 28, 2008 and claims a method of treating a vascular permeability-associated disease comprising administering a peptide inhibitor of p21-activated kinase. The specification of '681 patent provides Examples demonstrating *in vitro* binding, phosphorylation, and membrane permeability studies of p21-activated kinase in the presence of the peptide inhibitor; and *in vivo* permeability studies in mice. U.S. Pat. No. 7,446,183 (the '183 patent) was issued recently, on November 4, 2008, and claims a method of treating a growth hormone deficiency comprising administering a fusion protein that includes an agonist of a growth hormone receptor. The specification of the '182 patent provides Examples demonstrating synthesis of such fusion proteins, expression of such proteins in bacterial cells, *in vitro* bioassays of such fusion proteins, and metabolism of such fusion proteins in rats. Neither the '681 nor the '183 provide data on effects of the peptides

and/or proteins in humans, let alone provide any evidence that the recited proteins have any therapeutic effect *in vivo* in humans.

Appellants further note that the Examiner has based his enablement rejection on his allegation that the state of the art in protein therapy was not sufficiently predictable by the time of the filing of the present application (October 17, 2003). Nevertheless, the '818 patent issued from an application filed September 22, 1998 and the '329 patent issued from an application filed March 9, 1999, dates that precede the filing date of the present application as well as the filing date of the parent of the present application (April 21, 1999).

Appellants have provided evidence far exceeding the legal standard for enablement for method of treatment claims using peptides and/or proteins. Yet the Examiner continues to morph the enablement rejection, relying only arguments citing *potential* issues, each of which is refuted by the *evidence* presented by the Appellant. The Examiner has not articulated whether he disagrees with the legal standard set forth in *In re Brana* and as upheld in the issuing of numerous patents directed to peptide and/or protein therapy.

Appellants respectfully note that the Examiner has not articulated his reasons for holding the present application to a higher standard than that provided by the law.

In light of the above remarks, Appellants submit that claims 1, 15-17, 20, and 22-24 fully satisfy the enablement requirement under 35 U.S.C. § 112, first paragraph.

Claims 18 and 19 satisfy the enablement requirement under 35 U.S.C. § 112, first paragraph

Claims 18 and 19 depend from claim 17 (which recites that the chlorotoxin is selected from the group consisting of native chlorotoxin, synthetic chlorotoxin, and recombinant chlorotoxin) and are drawn to embodiments in which the neuroectodermal tumor is a glioma.

In addition to the arguments set forth above for claims 1, 15-17, 20, and 22-24, enablement for Claims 18 and 19 is also supported by data described in a Declaration by Alison M. O'Neill, M.D. (the "Declaration") filed by the Appellants on May 21, 2007 and included in the Evidence Appendix attached hereto. The Declaration describes data from human clinical

trials that shows, among other things, that chlorotoxin can be used to deliver a cytotoxic moiety to gliomas. For example, §§ **4-5** of the Declaration describes delivery of a cytotoxic moiety using chlorotoxin to high-grade gliomas including recurring high-grade gliomas by intracavitary administration. § **6** of the Declaration describes delivery of a cytotoxic moiety using chlorotoxin to a variety of tumors including malignant glioma using intravenous administration. § **7** describes the clinical improvement of one patient with malignant glioma to whom a chlorotoxin-cytotoxic moiety conjugate was delivered by intravenous administration.

In light of the above remarks, Appellants submit that claims 18 and 19 fully satisfy the enablement requirement under 35 U.S.C. § 112, first paragraph.

Claims appendix A

Pending claims

(As submitted in Supplemental Response filed March 30, 2009)

1. **(Previously Presented)** A method of delivering a cytotoxic moiety to a neuroectodermal tumor, comprising: administering a composition comprising an agent consisting of chlorotoxin fused to a cytotoxic moiety to an individual having a neuroectodermal tumor, such that the agent binds specifically to the tumor.

2-14. **Canceled**

15. **(Previously Presented)** The method of claim 1 wherein the chlorotoxin is fused to a cytotoxic moiety selected from the group consisting of gelonin, ricin, saporin, pseudomonas exotoxin, pokeweed antiviral protein, diphtheria toxin, and complement proteins.

16. **(Previously Presented)** The method of claim 1, wherein the neuroectodermal tumor is a tumor type is selected from the group consisting of ependymomas, medulloblastomas, neuroblastomas, gangliomas, pheochromocytomas, melanomas, peripheral primitive neuroectodermal tumors, small cell carcinoma of the lung, Ewing's sarcoma, and metastatic tumors in the brain.

17. **(Currently Amended)** The method of claim 15, wherein the chlorotoxin is selected from the group consisting of native chlorotoxin, synthetic chlorotoxin and recombinant chlorotoxin.

18. **(Previously Presented)** The method of claim 17, wherein the neuroectodermal tumor is a glioma.

19. **(Previously Presented)** The method of claim 18, wherein the glioma is selected from the group consisting of WHO grade IV: glioblastoma multiforms, WHO grade III:

anaplastic astrocytoma, WHO grade II: low grade, WHO grade I: pliocytic astrocytoma, oligodendrogliomas, gangliomas, meningiomas and ependymomas.

20. (**Presently Presented**) The method of claim 17, wherein the tumor is selected from the group consisting of ependymomas, medulloblastomas, neuroblastomas, gangliomas, pheochromocytomas, melanomas, peripheral primitive neuroectodermal tumors, small cell carcinoma of the lung, Ewing's sarcoma, and metastatic tumors in the brain.

21. (**Canceled**)

22. (**Previously Presented**) The method of claim 1 wherein the composition further comprises a pharmaceutically acceptable carrier.

23. (**Previously Presented**) The method of claim 1 wherein the composition is suitable for parenteral administration.

24. (**Currently Amended**) The method of claim ~~1~~23 wherein the parenteral administration is selected from the group consisting of intravenous, intramuscular, intrathecal and subcutaneous administration.

25-28. (**Canceled**)

Claims appendix B

Pending claims

(Assuming entrance of Amendment filed March 30, 2009)

1. **(Previously Presented)** A method of delivering a cytotoxic moiety to a neuroectodermal tumor, comprising: administering a composition comprising an agent consisting of chlorotoxin fused to a cytotoxic moiety to an individual having a neuroectodermal tumor, such that the agent binds specifically to the tumor.

2-14. **Canceled**

15. **(Previously Presented)** The method of claim 1 wherein the chlorotoxin is fused to a cytotoxic moiety selected from the group consisting of gelonin, ricin, saporin, pseudomonas exotoxin, pokeweed antiviral protein, diphtheria toxin, and complement proteins.

16. **(Previously Presented)** The method of claim 1, wherein the neuroectodermal tumor is a tumor type is selected from the group consisting of ependymomas, medulloblastomas, neuroblastomas, gangliomas, pheochromocytomas, melanomas, peripheral primitive neuroectodermal tumors, small cell carcinoma of the lung, Ewing's sarcoma, and metastatic tumors in the brain.

17. **(Previously Presented)** The method of claim 1, wherein the chlorotoxin is selected from the group consisting of native chlorotoxin, synthetic chlorotoxin and recombinant chlorotoxin.

18. **(Previously Presented)** The method of claim 17, wherein the neuroectodermal tumor is a glioma.

19. **(Previously Presented)** The method of claim 18, wherein the glioma is selected from the group consisting of WHO grade IV: glioblastoma multiforms, WHO grade III:

anaplastic astrocytoma, WHO grade II: low grade, WHO grade I: pliocytic astrocytoma, oligodendrogliomas, gangliomas, meningiomas and ependymomas.

20. (**Presently Presented**) The method of claim 17, wherein the tumor is selected from the group consisting of ependymomas, medulloblastomas, neuroblastomas, gangliomas, pheochromocytomas, melanomas, peripheral primitive neuroectodermal tumors, small cell carcinoma of the lung, Ewing's sarcoma, and metastatic tumors in the brain.

21. (**Canceled**)

22. (**Previously Presented**) The method of claim 1 wherein the composition further comprises a pharmaceutically acceptable carrier.

23. (**Previously Presented**) The method of claim 1 wherein the composition is suitable for parenteral administration.

24. (**Previously Presented**) The method of claim 23 wherein the parenteral administration is selected from the group consisting of intravenous, intramuscular, intrathecal and subcutaneous administration.

25-28. (**Canceled**)

Evidence appendix

Appellants had provided the following evidence during prosecution of the instant application:

(1) Abstract by Veisch *et al.* presented at the Nanotech 2006 Conference ("Abstract"). The Abstract was submitted by Appellants as Appendix A in a response to Office Action filed September 15, 2006 and was entered into the record in PAIR on the same date, as page 7 of the entry designated "Applicant Arguments/Remarks Made in Amendment." Entrance into the record was confirmed by the Examiner's reference to this Abstract on page of the Office Action mailed on November 21, 2006.

The Abstract is attached hereto on pages 29-30.

(2) Declaration by Alison M. O'Neill, M.D. and Exhibits A, B, C, and D. The Declaration and Exhibits A, B, C, and D were submitted along with a response to Office Action filed May 21, 2007 and was entered into the record in PAIR on May 25, 2007 as the entry designated "Rule 130, 131 or 132 Affidavits." Entrance into the record was confirmed by the Examiner's reference to this Declaration on page 2 of the advisory action mailed on June 27, 2007.

The Declaration is attached hereto on pages 31-33.

Exhibit A is attached hereto on pages 35-38.

Exhibit B is attached hereto on pages 39-45.

Exhibit C is attached hereto on pages 46.

Exhibit D is attached hereto on page 47.

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Tumor Paint: A chlorotoxin-based biomarker for intra-operative imaging of cancer foci

M. Veisch, S.B. Bahrami, P. Gabikian, R.G. Ellenbogen and J.M. Olson
Fred Hutchinson Cancer Research Center, US

Keywords:
cancer diagnosis, cancer trageting, chlorotoxin, near infrared imaging

Abstract:
Recent advances in molecular biology and nano-medicine have improved early tumor detection through recognition of molecules that are specifically expressed in malignant cells. This has the potential to specifically "paint" tumors with targeted molecular probes. In this view, we developed and characterized a near infrared (NIR) chlorotoxin-based probe to detect and paint cancer cells in vitro, in vivo and ex vivo. We demonstrate the exquisite delineation of malignant brain cells (glioma and medulloblastoma) from normal brain tissue after systemic administration of the probe in 2 mouse models. The medulloblastoma tumors were formed in the absence of surgical disruption of the blood brain barrier and their NIR signal were detected through intact skull and scalp. The broader utility of the probe was demonstrated through ex vivo and in vivo imaging of adenocarcinoma, rhabdomyosarcoma and spontaneous prostate cancer mice models. Results showed that it could specifically demarcate primary tumors and cognate lung and lymphatic metastases as small as 1.5 mm in diameter. Biodistribution and toxicity studies indicated favorable properties for advancement to human trials. This probe has potential to improve not only intraoperative tumor detection and resection but also diagnosis and imaging of various malignancies.

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Authors

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Nanotech 2006 Program - Tuesday May 9

Sessions Sunday Monday Tuesday Wednesday Thursday Authors Keywo

8:30 **Keynote - Sensors & Systems: MEMS & NEMS, MSM** Roon
Session chair: Elena Gaura, Coventry University, UK
8:30 Nano Electromechanical Devices: Opportunities and Challenges (invited)
R.T. Howe, *Stanford University, US* (speaker biography)
9:15 A Designer's Guide to CMOS MEMS (invited)
G.K. Fedder, S. Simone and N. Sarkar, *Carnegie Mellon University, US* (speaker biography)

8:30 **Keynote - Soft Nanotechnology: Characterization** Roon
Session chair: Fiona Case, Case Scientific, US
8:30 Colloidal Delivery Systems for Functional Food Design (invited)
K. Velikov, *Unilever R&D, UK* (speaker biography)
9:15 Characterization of Nanostructured Materials (invited)
S.K. Sinha, *University of San Diego, US* (speaker biography)

8:30 **Keynote - Nanotechnology for Cancer Prevention, Diagnosis and Treatment**
Session chair: Mansoor Amiji, Northeastern University
8:30 Challenges in Cancer Prevention, Diagnosis, and Therapy (invited)
J. Folkman, *Children's Hospital Boston, US* (speaker biography)
9:15 Delivery of Nano-medicine to Solid Tumors: Role of Tumor Physiology (invited)
R.K. Jain, *MGH, Harvard Medical School, US* (speaker biography)

8:30 **WCM 1 - Bulk MOS intrinsic models** Roon
Session chair: Xing Zhou, Nanyang Technological University, Singapore
8:30 Carrier Generation and Recombination Currents At Interface Traps In Surface-Potential-Based MOS Transistor Compact Models (invited)
C-T Sah and B.B. Jie, *University of Florida, US*
9:00 Symbolic charge-based MOSFET model (invited)
C. Galup-Montoro and M.C. Schneider, *Federal University of Santa Catarina, BR*
9:30 Theory and Modeling Techniques used in PSP Model (invited)
G. Gildenblat, X. Li, H. Wang, W. Wu, A. Jha, R. van Langevelde, A.J. Scholten, G.D.J. Smit and D.B Klaassen, *Pennsylvania State University, US*

8:30 **Clean and Controlled Environments - A4** Roon
Session chair: Paul Nesdore, Controlled Environments, US
8:30 Why Choose a Design/Build Contractor?
D. Kirkpatrick, *Western Environmental, US*
9:15 The Green Clean Laboratory
R.K. Schneider, *Clemson University, US*

8:30 **Clean and Controlled Environments - B4** Roon
Session chair: Patrice Galvin, Controlled Environments, US
8:30 Critical Cleaning Using CO2 Snow
R. Sherman, *Applied Surface Technologies, US*
9:15 Chemical Filtration Strategies for the Control of Airborne Molecular Contamination
B. Stanley, *Purafil, US*

8:30 **TechConnect Corporate Models and Needs** Roon

2/2

Attorney Docket No: 2006636-0064

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Sontheimer, *et al.*	Examiner:	Chen, Shin-Lin
Serial No.:	10/686,782	Art Unit:	1632
Filing Date:	October 17, 2003		
Title:	Diagnosis and Treatment of Neuroectodermal Tumors		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION UNDER 37 C.F.R. § 1.132

I, Alison M. O'Neill, M.D., declare as follows:

1. I am the Vice President, Medical Affairs at TransMolecular, Inc., Cambridge, Massachusetts, the assignee of United States patent application Serial No. 10/686,782, filed October 17, 2003, and entitled "Diagnosis and Treatment of Neuroectodermal Tumors". A copy of my curriculum vitae is attached hereto as **Exhibit A.**

2. I have reviewed the specification and claims of the above-referenced patent application as well as the Office Action mailed November 21, 2006, and understand that the Examiner has rejected all the pending claims for failing to comply with the enablement requirement. I further understand that the Examiner has questioned whether a chlorotoxin fused to a cytotoxic moiety (1) can pass through the blood-brain barrier in a subject, and (2) can be administered to provide therapeutic effect *in vivo*.

3. One purpose of the present Declaration is to confirm assertions made in the Specification that chlorotoxin fused to a cytotoxic moiety passes through the blood-brain barrier when administered to a subject, and has a therapeutic effect *in vivo*. Another purpose of this

Declaration is to confirm that binding of a chlorotoxin-cytotoxic moiety complex to neuroectodermal tissue correlates with therapeutic activity.

4. A synthetic version of chlorotoxin (TM-601) has been manufactured and covalently linked to iodine 131 (^{131}I-TM-601), a cytotoxic moiety. Preclinical studies and Phase I clinical trials have been completed, under my supervision, in patients with recurrent high-grade glioma. These studies demonstrated that intracavitary dosing of ^{131}I-TM-601 appears safe, minimally toxic, and binds malignant glioma with high affinity and for long durations. Some of the results obtained in this study have been reporting in A.N. Mamelak *et al.*, "Phase I Single-Dose Study of Intracavitary-Administered Iodine-131-TM-601 in Adults With Recurrent High-Grade Glioma", Journal of Clinical Oncology, August 1, 2006, 24: 3644-3650 ("Mamelak") (see **Exhibit B**). As of February 2007, out of the 18 patients that have received a single dose of ^{131}I-TM-601 in the Phase I trial, 5 survived 12 months or longer from recurrence; 2 survived more than 36 months from recurrence; and 1 patient remains alive (more than 4 years from recurrence). These results confirm the assertion made in the Specification that chlorotoxin with radioactive moieties selectively bind to gliomas and expose cells to high levels of radioactivity and can therefore be used to treat gliomas (see last sentences of Example 21 of the parent U.S. Pat. No. 5,905,027, which is incorporated by reference in the instant Application).

5. A Phase II trial of ^{131}I-TM-601 using higher doses of radioactivity and repeated intracavitary administrations to patients with high-grade glioma is underway, under my supervision. In the Dose Escalation Phase of this trial, patients received ^{131}I-TM-601 at 0.4mg/20mCi, repeated 3 times at 7 day intervals, ^{131}I-TM-601 at 0.6mg/30mCi, repeated 3 times at 7 day intervals, ^{131}I-TM-601 at 0.8mg/40mCi, repeated 3 times at 7 day intervals or ^{131}I-TM-601 at 0.8mg/40mCi, repeated 6 times at 7 day intervals. In the Randomized Phase of this trial, patients have received or are receiving ^{131}I-TM-601 at 0.8mg/40mCi, repeated 3 times or 6 times at 7 day intervals. **Exhibit C** is a graph showing the length of survival (determined in April 2007) for each patient enrolled in this Phase II trial, after treatment with ^{131} I-TM-601. As of April 2007, of the patients who had been on study for 6 months, 86% (31 out of 36 patients) remained alive, of the patients who had been on study for 9 months, 63% (17 out of 27 patients) remained alive, and of the patients who had been on study for 12-months, 45% (10 out of 22

patients) remained alive. These results demonstrate the therapeutic effect of a chlorotoxin-cytotoxic moiety complex *in vivo*.

6. In addition, enrollment has begun in a Phase I trial evaluating the biodistribution and safety of systemic delivery of ^{131}I-TM-601 to patients with recurrent or refractory primary solid tumors (including malignant glioma) with metastatic involvement (including brain metastases). In this trial, patients receive an intravenous dose of ^{131}I-TM-601 at 0.2mg/10mCi, and, if necessary, a second intravenous dose of ^{131}I-TM-601 at 0.4mg/20mCi, to allow for tumor localization. Patients whose tumor is localized then receive an intravenous treatment dose of ^{131}I-TM-601 at 0.6mg/30mCi. Preliminary results showed that intravenous administration of ^{131}I-TM-601 resulted in tumor-specific localization of ^{131}I-TM-601 in 5 out of 5 patients with malignant glioma, 1 out of 1 patient with prostate cancer, 1 out of 1 patient with Non-Small Cell Lung cancer, 1 out of 2 patients with metastatic melanoma, and 1 out of 1 patient with colon cancer. These results confirm that a chlorotoxin-cytotoxic moiety complex can reach the target site when administered to a patient, and further demonstrate that such a complex can pass through the blood-brain barrier to reach a tumor localized in the brain.

7. Furthermore, one patient with malignant glioma, who showed tumor-specific uptake of ^{131}I-TM-601 and who then received the intravenous treatment dose, was found to exhibit evidence of radiographic improvement (see **Exhibit D**, which shows a set of Magnetic Resonance (MRI) images recorded from that patient before and after ^{131}I-TM-601 treatment). Another patient with malignant glioma, who also showed tumor-specific uptake of ^{131}I-TM-601 and who then received the intravenous treatment dose, exhibited apparent clinical improvement in the absence of imaging improvement. These results demonstrate the therapeutic effect of a chlorotoxin-cytotoxic moiety complex *in vivo*.

8. The Specification teaches that binding of a chlorotoxin-cytotoxic moiety complex to neuroectodermal tumor tissues correlates with therapeutic activity *in vivo*. All the clinical trial data presented in the present Declaration confirm this correlation.

9. I, Alison M. O'Neill, declare that all statements made herein of my own knowledge are true and that these statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like are made punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patents that may issue thereon.

Alison M. O'Neill, M.D.

May 21, 2007

Date

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EDUCATION

1990	M.D., Pritzker School of Medicine University of Chicago, Chicago, Illinois
1983	A.B., Biological Sciences University of Chicago, Chicago, Illinois

POSTDOCTORAL TRAINING

1994 - 1996	Fellowship, Neuro-Oncology Memorial Sloan-Kettering Cancer Center New York, New York
1991 - 1994	Residency, Department of Neurology University of Michigan Hospitals Ann Arbor, Michigan
1990 - 1991	Internship, Department of Internal Medicine University of Chicago Hospitals, Chicago, Illinois

MEDICAL LICENSURE

2002 - present	Licensed physician, Commonwealth of Massachusetts
1996 - present	Licensed physician, State of Alabama
1994 - present	Licensed physician, State of New York
1991	Diplomate, National Board of Medical Examiners

PROFESSIONAL CERTIFICATIONS

2006	Recertified, American Board of Psychiatry and Neurology
1997	American Society of Neuroimaging (MRI and CT)
1996	Diplomate, American Board of Psychiatry and Neurology

EMPLOYMENT HISTORY

2006- present	Vice President, Medical Affairs TransMolecular, Inc. Cambridge, MA
2002- 2006	Assistant Professor of Neurology Harvard Medical School

Exhibit A

1996 - 2002	Assistant Professor, Department of Neurology University of Alabama at Birmingham
1996 - 2002	Associate Scientist, Neuro-Oncology Program UAB Comprehensive Cancer Center
1994 - 1996	Fellow, Department of Neurology Cornell University Medical College, New York, New York

HOSPITAL APPOINTMENTS

2002 – 2006	Assistant Neurologist Massachusetts General Hospital
1996 – 2002	Active Staff Physician, Neurology Service University of Alabama Hospitals
1996 – 2002	Consultant and Attending Neurologist, Birmingham VA Medical Center
1996 – 2002	Staff Physician Cooper Green Hospital, Birmingham, AL

PROFESSIONAL SOCIETIES

1997 – present	Society for Neuro-Oncology
1992 – present	American Academy of Neurology

HONORS AND AWARDS

1994 - 1995	American Cancer Society Clinical Oncology Fellowship Memorial Sloan-Kettering Cancer Center
1990	Alpha Omega Alpha Honor Medical Society University of Chicago Pritzker School of Medicine
1983	Dudley Medal, University of Chicago
1979 - 1983	Dudley Scholar, University of Chicago

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JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Phase I Single-Dose Study of Intracavitary-Administered Iodine-131-TM-601 in Adults With Recurrent High-Grade Glioma

Adam N. Mamelak, Steven Rosenfeld, Richard Bucholz, Andrew Raubitschek, L. Burt Nabors, John B. Fiveash, Sui Shen, M.B. Khazaeli, David Colcher, An Liu, Medhat Osman, Bart Guthrie, Susan Schade-Bijun, Diana M. Habliz, Vernon L. Alvarez, and Matthew A. Gonda

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Submitted December 21, 2005; accepted May 26, 2006.

Supported by TransMolecular Inc, Birmingham, AL.

Presented in part at the 20th International Conference, Larnaka, Cyprus, June 30-July 2, 2003; and at the 8th Annual Meeting of the Society for Neuro-Oncology, Keystone, CO, November 13-16, 2003.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/06/2422-3644/$20.00

DOI: 10.1200/JCO.2005.05.4699

Abstract

Purpose
TM-601 binds to malignant brain tumor cells with high affinity and does not seem to bind to normal brain tissue. Preclinical studies suggest that iodine-131 (^{131}I) –TM-601 may be an effective targeted therapy for the treatment of glioma. We evaluated the safety, biodistribution, and dosimetry of intracavitary-administered ^{131}I-TM-601 in patients with recurrent glioma.

Patients and Methods
Eighteen adult patients (17 with glioblastoma multiforme and one with anaplastic astrocytoma) with histologically documented recurrent glioma and a Karnofsky performance status of ≥ 60% who were eligible for cytoreductive craniotomy were enrolled. An intracavitary catheter with subcutaneous reservoir was placed in the tumor cavity during surgery. Two weeks after surgery, patients received a single dose of ^{131}I-TM-601 from one of three dosing panels (0.25, 0.50, or 1.0 mg of TM-601), each labeled with 10 mCi of ^{131}I.

Results
Intracavitary administration was well tolerated, with no dose-limiting toxicities observed. ^{131}I-TM-601 bound to the tumor periphery and demonstrated long-term retention at the tumor with minimal uptake in any other organ system. Nonbound peptide was eliminated from the body within 24 to 48 hours. Only minor adverse events were reported during the 22 days after administration. At day 180, four patients had radiographic stable disease, and one had a partial response. Two of these patients further improved and were without evidence of disease for more than 30 months.

Conclusion
A single dose of 10 mCi ^{131}I-TM-601 was well tolerated for 0.25 to 1.0 mg TM-601 and may have an antitumoral effect. Dosimetry and biodistribution from this first trial suggest that phase II studies of ^{131}I-TM-601 are indicated.

J Clin Oncol 24:3644-3650. © 2006 by American Society of Clinical Oncology

INTRODUCTION

Despite aggressive efforts, the prognosis for survival from malignant glioma has not significantly improved in the last 20 years.[1-5] The 5-year survival for glioblastoma remains approximately 3%, and the 2-year survival is approximately 8.2%.[2] TM-601 is a synthetic version of a peptide (chlorotoxin) found in the venom of the giant yellow Israeli scorpion *Leiurus quinquestriatus*.[6] This 36–amino acid peptide has been explored[7,8] as a candidate for targeting gliomas. TM-601 crosses blood-brain and tissue barriers[7] and binds to a phosphatidyl inositide, a phosphorylated lipid on lamellipodia of tumor cells.[8] Preclinical studies demonstrated the stability, safety, efficacy, and lack of immunogenicity of radioiodinated TM-601. We performed a phase I study to evaluate the safety, biodistribution, and dosimetry of intracavitary iodine-131 (^{131}I) –TM-601 in adult patients with recurrent high-grade glioma.

PATIENTS AND METHODS

Preparation of ^{131}I-TM-601

TM-601 (lyophilized, sterile, and pyrogen free) was radiolabeled with 10 mCi ^{131}I via the Iodogen bead method[10] at the clinical site and used within 78 hours (typically < 2 hours). Release specifications required less than 5% free iodine (by instant thin-layer chromatography) and no pyrogenicity.

Exhibit B

Patients and Treatment Protocol

This study was performed in accordance with the Declaration of Helsinki and with the approval of the US Food and Drug Administration and of the institutional review boards at each participating site. Informed consent was obtained from each patient before participation.

Adult (> 18 years) patients with histologically documented supratentorial malignant glioma, a Karnofsky performance status (KPS) of ≥ 60%, and a life expectancy of at least 3 months were eligible for this trial.[13] Patients were required to have unifocal tumors that progressed at the site of original disease after standard of care. Additional inclusion criteria included tumor cross-sectional diameter of less than 6 cm; no direct communication with the ventricle; no previous immunotherapy, gene therapy, implantable chemotherapy, or stereotactic radiosurgery; and at least 6 weeks from the last dose of nitrosourea-containing chemotherapy. Eligible patients underwent tumor debulking surgery; pathologic confirmation of high-grade glioma was performed for all patients. During surgery, a ventricular access device (Rickham or Ommaya reservoir) was placed in the tumor cavity. Patients recovered from surgery for 14 to 28 days before undergoing treatment with the study drug. This waiting period was chosen to avoid confounding between neurologic deficits after surgery and adverse events attributed to the study drug. Patients were excluded from the trial if it was deemed by the investigator that they suffered a surgical complication that made proceeding with treatment unsafe or if the KPS was less than 60%.

Magnetic Resonance Imaging

Details of all imaging methods are reported elsewhere.[12] Briefly, postoperative magnetic resonance imaging (MRI) scans were acquired for each patient before ^{131}I-TM-601 injection. Sequences included 3-mm thick coronal, axial, and sagittal T1-weighted images (with and without gadolinium contrast), axial and coronal T2, axial fluid attenuated inversion recovery (FLAIR), and three-dimensional spoiled gradient echo images. Additional images were acquired at 22 days after injection and again at 3 and 6 months after treatment. The 3-week scan was performed to assess for any early inflammatory changes potentially related to the peptide, whereas later scans were performed for surveillance of tumor status.

Injection of Radiolabeled Peptide

Patients received supersaturated potassium iodide (300 mg/kg) 1 day prior and 3 days after administration of ^{131}I-TM-601 to block uptake of ^{131}I by the thyroid gland. Patency of the venous access device was evaluated by injection of 0.5 to 1 mCi of ^{111}Indium-diethylenetriamine pentaacetate (^{111}In-DTPA) followed by gamma camera imaging of the head. Serial images were acquired every 2 minutes for 16 minutes to determine whether the ^{111}In-DTPA was leaking from the cavity site. The leakage was measured by total counts in a selected region of interest over the study period. If more than 30% of the ^{111}In-DTPA had leaked from the cavity site, administration of the study drug was aborted.

Next, patients received 25% of the total dose via injection into the venous access device, were observed for 5 minutes, and then received the remainder of the study dose. Patients were monitored during drug infusion and re-evaluated on a daily basis during the immediate (days 0 to 8) postinfusion period; patients were seen again at day 22 and then observed for up to 180 days. Biodistribution and elimination were determined by urine and blood measures of radioactivity. Twenty-four hour urine collections were performed over days 1 to 2, 2 to 3, 3 to 4, and 4 to 6 or 8, with an aliquot from each 24-hour period used to determine the average amount of excreted radioactivity during that time period. Blood samples were collected 1, 2, and 4 hours after the completion of the infusion on days 2, 3, and 4 and at the time of imaging on day 6 or 8.

Gamma Camera Imaging

Cobalt-57 transmission scan. A cobalt-57 transmission scan with and without the patient was used to obtain attenuation correction factors for total-body image quantification, as previously described.[22]

Whole-body and two-dimensional brain single photon emission computed tomography (SPECT) scans. After intracavitary injection of ^{131}I-TM-601, anterior and posterior whole-body planar images and two-dimensional SPECT scans were acquired as described.[12] A 20-mL calibrated ^{131}I source (approximately 100 μCi) was placed 10 cm from the feet of the patient within the field of view. Subsequent images were acquired on days 1, 2, and 3 and between 5 and 8 days after injection.

Study Design and Statistical Methods

The goals of this study were primarily to evaluate the safety, biodistribution, and dosimetry of a single dose of intracavitary ^{131}I-TM-601 infused into the tumor resection cavity. Because TM-601 had never been administered to humans and the toxic effects and dose-limiting toxicities (DLTs) of ^{131}I are well documented, a standard trace dose of 10 mCi ^{131}I was used for imaging as required by the US Food and Drug Administration before radioactivity dose-escalation trials. Estimates of the number of surface receptors for TM-601 on glioma cells indicated that peptide doses in the range of 0.1 to 1.0 mg were adequate to saturate all binding sites.[9] Thus, three peptide doses were chosen for dose-escalation studies, with the amount of radioactivity fixed at 10 mCi. This design was requested and approved by the US Food and Drug Administration.

Preliminary assessment of antitumor effect was a secondary end point. Six patients were enrolled onto one of three sequential dosing panels (panel 1, 0.25 mg of TM-601; panel 2, 0.50 mg of TM-601; and panel 3, 1.00 mg of TM-601), each radiolabeled with 10 mCi (± 10%) of ^{131}I. Treatment within a dosing panel would have been interrupted if two or more of the initial three patients experienced a DLT (grade 3 or higher according to the National Cancer Institute Common Toxicity Criteria version 3.0 and graded as at least probably related to treatment). Dose escalation similarly would have been interrupted if two or more DLTs occurred within a single dosing panel. If two patients at a given dose experienced a DLT, the previous dose level would have been identified as the maximum tolerated dose. Every patient was observed clinically for 180 days after treatment. All efficacy and safety analyses were performed on the intent-to-treat cohort of all patients who received a single dose of intracavitary ^{131}I-TM-601.

Radiation Dosimetry

The tissue uptake, clearance, and dosimetry of ^{131}I-TM-601 for the whole body, normal organs, and brain were determined based on five sequential, quantitative, whole-body gamma camera images as previously detailed.[15] Radiation to normal organs was calculated using the MIRDOSE III program (free software; Oak Ridge Laboratories, Oak Ridge, TN) based on the reference man.[16] Radiation dose of ^{131}I to the tissue surrounding the resection cavity was evaluated based on SPECT images. The counts in the SPECT images were converted to μCi of ^{131}I based on a calibrated imaging standard. The distribution of ^{131}I was converted to the radiation dose rate distribution using dose convolution with electron and photon dose kernel. Radiation doses to the tumor resection cavity were estimated to within 2 cm of tumor margins because most recurrences occur within this distance.[14,16]

Radioactivity concentrations in blood and urine were determined using a gamma well counter calibrated with a ^{131}I standard. For blood, total radioactivity was calculated based on the area under the radioactivity-time curve, with the typical peak (μCi/mL) at 4 hours. Cumulated activity during the 0- to 4-hour window was determined by trapezoid integration, and cumulated activity after more than 4 hours was fitted with a monoexponential curve. Marrow-to-blood ratio[17] was assumed to be 0.75 because of the small peptide size. Patient-specific marrow dose was estimated based on the electron radiation from the blood, the photon radiation from the remaining body and tumor cavity, and the patient's body weight.[16]

Histochemical Staining

A tissue sample was obtained from each patient during surgery. Each specimen was subjected to immunohistochemical staining to test for TM-601 binding. Staining followed the method of Lyons et al[10] with few modifications.

RESULTS

Nineteen patients with recurrent high-grade glioma were enrolled onto the study; 18 had glioblastoma multiforme (GBM), and one had anaplastic astrocytoma. One GBM patient was excluded after surgery

because of a diagnosis of previously undetected hepatitis C. The demographics of the patient population are listed in Table 1. All patients received at least one dose of study medication.

For unplanned reasons, two patients assigned to the 0.50-mg dose panel and one patient assigned to the 1.00-mg panel received a second dose of study medication. In one of these patients, SPECT images indicated that the first injection was accidentally delivered subcutaneously and did not enter the resection cavity. Calculated radiation doses to normal organs after this subcutaneous injection were determined to be clinically insignificant. This patient received a second injection of 10 mCi ^{131}I-TM-601 into the reservoir, confirmed by subsequent SPECT images. Two other patients received a second dose on a compassionate use basis, with approval from the US Food and Drug Administration, at 12 and 19 weeks after initial treatment. Survival from time of injection for all patients is shown in Figure 1. Two patients demonstrated a small amount of ^{111}In-DTPA leakage into the ventricles and spinal fluid pathways. Radiation dose estimates suggest that the radiation dose of ^{131}I-TM-601 to the spine was in a range thought to be clinically insignificant (2.83 Gy and 3.78 Gy). In these patients, the treating physician determined that administration of 10 mCi ^{131}I-TM-601 was still appropriate for this study.

Radiation Dosimetry

Radiation doses to normal organs were clinically insignificant (Table 2). In contrast, the mean radiation dose to within 2 cm of the cavity wall was 0.81 Gy/mCi (median, 0.49 Gy/mCi), and the dose ranged from 0.12 to 2.75 Gy/mCi (Table 2). Furthermore, the biologic half-life of ^{131}I-TM-601 in the tumor cavity margin was longer than in any other organ, indicating long-term retention of the drug in and around the injection site (Table 2). The median biologic half-life in cavity margin was 70 hours (range, 32 to 193 hours), 80 hours (range, 25 to 86 hours), and 55 hours (range, 41 to 62 hours) for patients receiving 0.25, 0.50, and 1.0 mg of peptide, respectively.

Table 1. Patient Characteristics

Characteristic	Overall	Panel 1	Panel 2	Panel 3
Age, years				
Mean	47.2	44.5	46.3	50.7
Standard deviation	10.9	14.7	9.8	10.7
Karnofsky performance status, %				
Mean	82.8	83.3	81.7	83.3
Standard deviation	11.3	15.1	11.7	8.2
Tumor location, No. of patients				
Frontal lobe	10	6	0	4
Temporal lobe	5	0	4	1
Parietal lobe	3	0	2	1

The biologic half-life, radiation dose per unit of injection dose (Gy/mCi), and radiation dose (Gy) for ^{131}I-TM-601 within the 2-cm tumor cavity wall are listed for each patient in Table 3. These data indicate a slightly longer half-life and higher radiation dose for patients receiving 0.50 mg of peptide compared with the other groups, although this difference did not reach statistical significance. ^{131}I-TM-601 localized to and remained primarily concentrated in and around the patients' surgical cavity for all 5 days that the patients were imaged (a typical image is shown in Fig 2).

Patient Follow-Up, Toxicity, and Response to Therapy

Eleven patients completed the 180-day observation period. There were no DLTs related to treatment during the initial 22-day observation period and no clinically significant acute adverse events during infusion of ^{131}I-TM-601 at any dose level. The majority of events reported were mild to moderate in nature. There were no grade 3 or 4 toxicities related to the study drug or method of administration in the immediate and/or long-term follow-up period. There were 88 grade 1 and 90 grade 2 toxicities. There were no patient complaints related to the study drug or method of administration.

Four patients had serious adverse events possibly or probably related to study medication reported within 22 days of administration (Table 4). Additional serious adverse events reported beyond the initial 22-day observation period included one patient with generalized seizure and increased confusion; one patient with pneumonia; one patient with somnolence, ventricular dilation, and cerebral hematoma; and one patient with headache, dysarthria, and instability. The administration of a second dose of study medication was not associated with any serious adverse events, although these events were not formally included in the toxicity evaluations because of the long time interval (12 and 19 weeks) between drug administrations.

Over the course of the 180-day observation period, there were seven deaths. Two patients in panel 2 with GBM have survived more than 30 months. Median survival time was 25.7 weeks for patients in panel 1 (0.25-mg dose), 77.6 weeks for patients in panel 2 (0.50-mg dose), 23.6 weeks for patients in panel 3 (1.00-mg dose), and 27.0 weeks for patients in all three dosing groups (Table 5). Histochemistry of the tumor tissue from all patients stained intensely positive for TM-601, as represented in Figures 3A to 3C.

Radiographic Changes

Tumor volume measurements were available for 16 patients at baseline (within 48 hours of surgery), 16 patients at 22 days after treatment, 16 patients at 90 days after treatment, and five patients at 180 days after treatment. All but one patient had evidence of residual enhancing disease on baseline scans. The mean baseline residual T1 enhancing tumor volume was 28 ± 28 mL (range, 0 to 72.15 mL). On day 22 after treatment, this volume had increased to a mean of 31.8 ± 32.7 mL (range, 1.8 to 114.2 mL). The tumor volumes decreased by 10.8% in one patient and 76.7% in another patient, were unchanged in nine patients, and increased in three patients (four patients were not assessable). This translated into a radiographic interpretation of stable disease in 12 patients and progressive disease in four patients (two patients were not assessable at this time point; Table 4). For 16 patients with radiographic follow-up available at 90 days, a stable response was observed in seven patients, and progressive disease

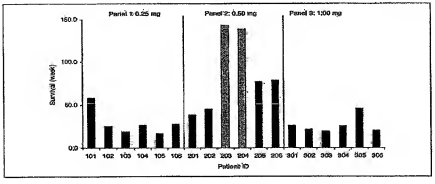

Fig 1. Individual patient survival. Length of survival for each patient (as of November 2003) after treatment with 10 mCi iodine-131-TM-601 measured in weeks.

was observed in nine patients (two patients were not assessable at this time point). Long-term follow-up was available for six patients, with one patient showing a partial response (defined as at least a 50% decrease under baseline with no new lesions), four with stable disease, and one with progressive disease. Two patients (one with stable disease and one with partial response) went on to achieve a complete radiographic response (defined as complete absence of demonstrable contrast enhancement on T1-weighted MRI) without evidence of disease for 32 and 30 months. The patients (patients 203 and 204) were females and were ages 40 and 42 years. Both patients had parietal lobe GBM (one left hemisphere and one right hemisphere), a KPS of 90% after resection, and minimal residual enhancement on postoperative MRIs. Neither patient received a second dose of ^{131}I-TM-601. An example of stable disease (patient 204) is demonstrated in Figures 3D and 3E.

DISCUSSION

In this first human trial, treatment of patients with recurrent high-grade glioma with a single intracavitary dose of ^{131}I-TM-601 was well tolerated to the dose of 1.0 mg TM-601 radiolabeled with 10 mCi of ^{131}I. Few adverse effects occurred during the initial 22-day observation period, which suggests the dosing level of peptide used in this study is safe and well tolerated. It is unlikely that the doses of ^{131}I contributed to the adverse events because the doses were far below expected toxicity ranges.[20] The adverse events that did occur were considered unremarkable in this patient population. Three patients received two doses without any significant adverse events, preliminarily demonstrating that repeated administration of ^{131}I-TM-601 may be safe.

Biodistribution data of ^{131}I-TM-601 indicated that this radiopeptide rapidly penetrated through the cavity wall with, on average, 79% of the radioactivity leaving the region of cavity within 24 hours after administration. The majority of the remaining radioactivity stayed tightly localized to the tumor cavity and surrounding regions, suggesting discrete binding to the tumor. The amount of uptake and radiation

Table 2. Organ Dose and Half-Life

Organ	Dose (Gy/mCi) Mean	Dose (Gy/mCi) Range	Biologic Half-Life (hours) Mean	Biologic Half-Life (hours) Range
Stomach	0.007	0.002-0.013	25	17-40
Thyroid	0.053	0.008-0.229	NA	
Marrow, blood half-life	0.003	0.001-0.004	29	9-41
Tumor cavity wall	0.91	0.12-2.75	69	26-193

Abbreviation: NA, not available.

Table 3. Individual Patient Dosimetry and Half-Life of ^{131}I-TM-601 at Tumor Cavity Wall

Dose and Patient No.	Biologic Half-Life (hours)	Average Biologic Half-Life (hours) Mean	Average Biologic Half-Life (hours) Range	Dose per Unit Injection/Dose (Gy/mCi)	Average Dose (Gy) Mean	Average Dose (Gy) Range
0.50 mg		79.8	25.1-95.4		7.29	2.80-12.96
201	86.4			0.906		
202	78.9			0.376		
203	55.2			1.168		
204	25.1			0.951		
205	80.8			0.957		
206	85.5			0.263		

Abbreviation: ^{131}I, iodine-131.

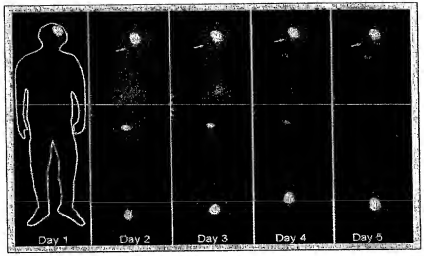

Fig 2. Gamma camera scans. Anterior gamma camera scans for patient 104. Intense localization is seen in the surgical cavity as late as 5 days after infusion. This result was confirmed on brain single photon emission computed tomography scans and was observed uniformly in all patients. Note the location of hot spots outside the surgical cavity (arrows).

doses in the stomach, kidneys, spleen, and bladder were much lower compared with those reported in the literature for other modalities.[21,22] There was no observable uptake of ^{131}I-TM-601 in the small or large intestine at any time in any patient, suggesting that the excretion route of ^{131}I-TM-601 is mainly through the urinary tract. Uptake of ^{131}I-TM-601 in the thyroid was greater than in other solid organs but still far below toxic levels, which are reported to be in the range of 100 Gy to cause hypothyroidism in greater than 50% of patients.[23,24]

Table 4. Adverse Events

Event	Severe Adverse Event Grade	No. of Occurrences
[redacted]		
Events likely related to study drug		
Infection	1	1
Paresis	1	3
Cerebral edema	1	1

*One patient each had candidal infection and herpes zoster.

Immunohistochemical studies in normal human tissues have failed to demonstrate TM-601 binding in normal thyroid gland,[7] which is consistent with our observation.

A detailed analysis of the imaging of this drug in the brain based on a subset of nine patients has been published,[12] indicating that ^{131}I-TM-601 diffuses into the brain at distances far greater than observed for antibodies and other large molecules.[25] These observations

Table 5. Tumor Response and Survival

Patient No.	Tumor Response by MRI			Survival Time (weeks)
	Day 22	Day 90	Day 180	
[redacted]				
102	SD	PD	NA	26.0
[redacted]				
104	SD	SD	SD	28.4
[redacted]				
106	SD	SD	NA	27.2
[redacted]				
202	NA	PD	NA	44.9
[redacted]				
204	SD	SD	PR	133.7
[redacted]				
206	SD	PD	NA	78.6
[redacted]				
302	SD	PD	NA	21.9
[redacted]				
304	SD	PD	NA	25.4
[redacted]				
306	PD	NA	NA	20.1

Abbreviations: MRI, magnetic resonance imaging; PD, progressive disease; NA, not available; SD, stable disease; PR, partial response.
*Received second therapeutic dose of iodine-131-TM-601 on a compassionate use basis.

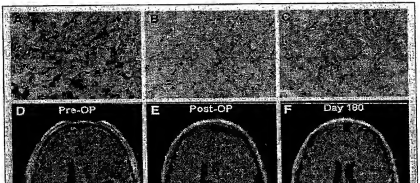

Fig 3. Histology and radiographic response. Representative histology of a sample from a patient with glioblastoma multiforme (patient 2004) is shown. Frozen sections were fixed and stained with biotinylated peptides. Streptavidin-horseradish peroxidase and diaminobenzidine were used for detection as indicated by the intense brown staining with biotinylated TM-601 in panel A. (A) Biotinylated TM-601 (10 μmol/L). (B) No peptide-negative control. (C) Hematoxylin and eosin stain. Radiographic response of this patient is shown in panels D, E, and F, indicating stable disease at 180 days after treatment.

suggest that TM-601 may be a useful means to deliver focused radiotherapy to patients with glioma. Limited by current state of the art imaging techniques and because of the nonuniform microscopic distribution of ^{131}I-TM-601 and residual tumor cells, the current macroscopic radiation dose calculations based on imaging may not accurately represent the actual radiation dose delivered to tumor cells.[24]

In two GBM patients receiving 0.5 mg TM-601 plus 10 mCi ^{131}I (± 10%), a complete radiographic response was observed. These two patients are still alive 37 and 39 months after surgery (as of March 2006) even with this low dose of peptide and expected subtherapeutic level of radiation. Of note, these patients were slightly younger than the average patient in the study (ages 40 and 42 years) but were otherwise quite representative of the remainder of the study cohort. Thus, although we acknowledge that confounding factors, such as patient age, tumor size, extent of resection, and KPS, may have contributed to this result,[27] the responses certainly suggest that further investigation of this minimally toxic agent is warranted.

Intracavitary-administered ^{131}I-TM-601 is simple to deliver, well tolerated, remains highly localized to the treatment site, and preliminarily seems safe for repeated injections. Recently, a phase II trial has been initiated using escalating peptide and radiation doses with multiple injections for patients with high-grade glioma.

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Authors' Disclosures of Potential Conflicts of Interest

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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Other: Andrew Raubitschek (method design and radiopharmaceutical administration), Vernon L. Alvarez (method design and radiopharmaceutical administration)

Status as of April 4, 2007

6 month survival = 31/36 (86%)
9 month survival = 17/27 (63%)
12 month survival = 10/22 (45%)

- Confidential -

Exhibit C

IV Injection in Malignant Glioma
Pt 01-007: Pretreatment MRI (1/8/2007)
- Confidential -
3 weeks following 30 mCi dose (2/22/2007)

Exhibit D

Related proceedings appendix

None.

Conclusion

Appellants conclude with the belief that claims 1, 15-20, and 22-24 are fully enabled. Allowance of the pending claims is earnestly requested.

Please charge any additional fees that may be associated with this matter, or credit any overpayments, to our Deposit Account No.: 03-1721.

Respectfully submitted,

/Brenda Herschbach Jarrell/

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